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(54) Genetic compositions and methods

(57) This invention provides nucleic acid segments derived from the human genome, including polymorphic sites. Allele-specific primers and probes hybridizing to these sites and to regions flanking these sites are also provided. This invention further provides methods of analyzing a nucleic acid from an individual or a group of individuals. The nucleic acids, primers, and probes are useful tools for applications including forensics, paternity testing, medicine, e.g., the coorrelation of polymorphisms with phenotypic traits, and genetic analysis, e.g., genetic mapping of such phenotypic traits.

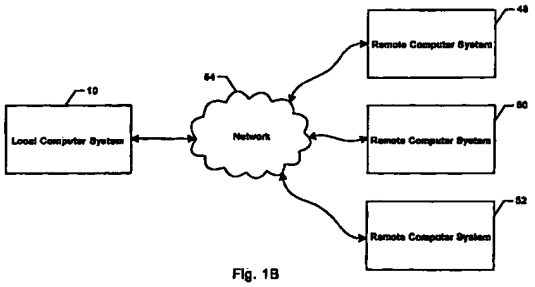

Fig. 1B

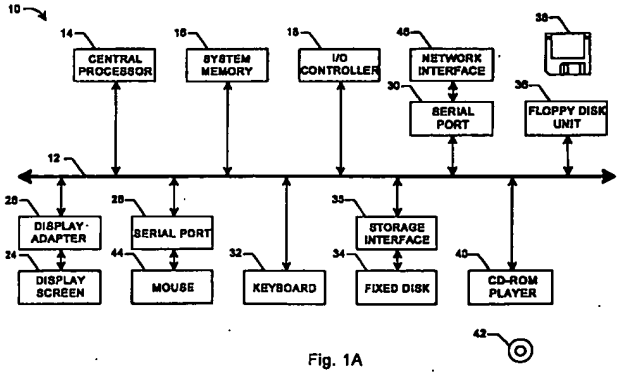

Fig. 1A

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Vol. 182 (1990)). Where the protein is secreted, it can be isolated from the supernatant in which the host cell is grown. Where the protein is not secreted, the protein can be isolated from a lysate of the host cells.

[0066] This invention further provides transgenic nonhuman animals capable of expressing an exogenous variant gene and/or having one or both alleles of an endogenous variant gene inactivated. Expression of an exogenous variant gene is usually achieved by operably linking the gene to a promoter and optionally an enhancer, and microinjecting the construct into a zygote. (See, e.g., Hogan et al., "*Manipulating the Mouse Embryo, A Laboratory Manual,*" Cold Spring Harbor Laboratory.) Inactivation of endogenous variant genes can be achieved by forming a transgene in which a cloned variant gene is inactivated by insertion of a positive selection marker. (See, e.g., Capecchi, *Science* 244, 1288-1292 (1989)). The transgene is then introduced into an embryonic stem cell, where it undergoes homologous recombination with an endogenous variant gene. Mice and other rodents are preferred animals. Such animals provide useful drug screening systems.

[0067] In addition to substantially full-length polypeptides expressed by variant genes, this present invention also includes biologically active fragments of the polypeptides, or analogs thereof, including organic molecules which simulate the interactions of the peptides. Biologically active fragments include any portion of the full-length polypeptide that confers a biological function on the variant gene product, including ligand and antibody binding. Ligand binding includes binding by nucleic acids, proteins or polypeptides, small biologically active molecules, or large cellular structures.

[0068] Polyclonal and/or monoclonal antibodies that specifically bind to variant gene products but not to corresponding prototypical gene products are also provided by this invention. Antibodies can be made by injecting mice or other animals with the variant gene product or synthetic peptide fragments thereof Monoclonal antibodies are screened as are described, for example, in Harlow & Lane, *Antibodies, A Laboratory Manual,* Cold Spring Harbor Press, New York (1988); and Goding, *Monoclonal antibodies, Principles and Practice* (2d ed.) Academic Press, New York (1986). Monoclonal antibodies are tested for specific immunoreactivity with a variant gene product and lack of immunoreactivity to the corresponding prototypical gene product. These antibodies are useful in diagnostic assays for detection of the variant form, or as an active ingredient in a pharmaceutical composition.

V. KITS

[0069] This invention further provides kits comprising at least one allele-specific oligonucleotide as described above. Preferably, the kits contain one or more pairs of allele-specific oligonucleotides hybridizing to different forms of a polymorphism. In some kits, the allele-specific oligonucleotides are provided immobilized to a substrate. For example, the same substrate can comprise allele-specific oligonucleotide probes for detecting at least 10, 100 or all of the polymorphisms shown in TABLE 1. Optional additional components of the kit include, for example, restriction enzymes, reverse-transcriptase or polymerase, the substrate nucleoside triphosphates, means used to label (for example, an avidin-enzyme conjugate and enzyme substrate and chromogen where the label is biotin), and the appropriate buffers for reverse transcription, PCR, or hybridization reactions. Generally, the kit also contains instructions for carrying out the methods.

VI. Computer Systems For Storing Polymorphism Data

[0070] Fig. 1A depicts a block diagram of a computer system 10 suitable for implementing the present invention. Computer system 10 includes a bus 12 which interconnects major subsystems such as a central processor 14, a system memory 16 (typically RAM), an input/output (I/O) controller 18, an external device such as a display screen 24 via a display adapter 26, serial ports 28 and 30, a keyboard 32, a fixed disk drive 34 via a storage interface 35 and a floppy disk drive 36 operative to receive a floppy disk 38, and a CD-ROM (or DVD-ROM) device 40 operative to receive a CD-ROM 42. Many other devices can be connected such as a user pointing device, e.g., a mouse 44 connected via serial port 28 and a network interface 46 connected via serial port 30.

[0071] Many other devices or subsystems (not shown) may be connected in a similar manner. Also, it is not necessary for all of the devices shown in Fig. 1A to be present to practice the present invention, as discussed below. The devices and subsystems may be interconnected in different ways from that shown in Fig. 1A. The operation of a computer system such as that shown in Fig. 1A is well known. Databases storing polymorphism information according to the present invention can be stored, e.g., in system memory 16 or on storage media such as fixed disk 34, floppy disk 38, or CD-ROM 42. An application program to access such databases can be operably disposed in system memory 16 or sorted on storage media such as fixed disk 34, floppy disk 38, or CD-ROM 42.

[0072] Fig. 1B depicts the interconnection of computer system 10 to remote computers 48, 50, and 52. Fig. 1B depicts a network 54 interconnecting remote servers 48, 50, and 52. Network interface 46 provides the connection from client computer system 10 to network 54. Network 54 can be, e.g., the Internet. Protocols for exchanging data via the Internet and other networks are well known. Information identifying the polymorphisms described herein can be transmitted across network 54 embedded in signals capable of traversing the physical media employed by network 54.